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Hepatic drug metabolism in bushbabies (*Galago crassicaudatus* and *Galago senegalensis*) and tree shrews (*Tupaia glis*)

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The rates and substrate specificities of hepatic drug-metabolizing enzymes vary considerably from species to species. Wide species variations in both qualitative and quantitative aspects of drug metabolism have been described [see Refs. 1–3]. The examination of hepatic microsomal metabolic pathways in various species has been motivated, in part, by the need for experimental models applicable to the evaluation of drug metabolism in man. Accordingly, much attention has been focused on hepatic drug metabolism in primates [see Ref. 3], especially those most closely related to man. However, relatively little is known about hepatic drug-metabolizing enzyme systems in the more primitive primates, the prosimians. In this communication, some of the characteristics of hepatic microsomal mixed function oxidases in the prosimian species, lesser and greater bushbabies (*Galago senegalensis* and *Galago crassicaudatus*) and tree shrews (*Tupaia glis*), are described. For the sake of comparison, similar data obtained from rats are also presented.

Male Sprague–Dawley rats (225–250 g) were obtained from Zivic–Miller Laboratories, Pittsburgh, PA. Sexually mature tree shrews (130–175 g), lesser bushbabies (160–240 g) and greater bushbabies (800–1100 g) were purchased from Primate Imports of New York and allowed several weeks of acclimation prior to being killed. Primates were housed individually on 0.3 m^3 steel cages and fed monkey chow mixed with bananas. All animals received food and tap water *ad lib.*

Animals were anesthetized with ether between 9.00 and 10.00 a.m. and the livers were removed immediately. Acute exposure to ether had no effects on hepatic drug metabolism. Livers were homogenized in cold 1.15% potassium chloride and centrifuged at 9000 *g* for 20 min in a Sorvall refrigerated centrifuge. Aliquots of the supernatant fraction were removed for enzyme assays, and the rest was centrifuged at 105,000 *g* for 60 min in a Beckman preparative ultrocentrifuge. Microsomal pellets were resuspended in 1.15% potassium chloride containing 0.05 M Tris–HCl buffer (pH 7.4) at a concentration of 3–4 mg protein/ml. All steps in the preparation of microsomes were carried out with the tissue kept at 0–4°. Microsomal cytochrome P-450 was measured as described by Omura and Sato [4]. NADPH-cytochrome *c* reductase activity was assayed by the method of Phillips and Langdon [5] and microsomal protein was determined as described by Lowry *et al.* [6].

Ethylmorphine or aminopyrine demethylation and aniline hydroxylation were assayed as the amounts of formaldehyde [7] or para-aminophenol [8] formed, respectively, by the 0.5 ml liver 9000 *g* supernatant fraction (equivalent to 200 mg/ml) incubated with glucose-6-phosphate (9.0 μmoles), NADP (2.08 μmoles), $MgSO_4$ (24.2 μmoles), Tris–HCl buffer (0.05 M; pH 7.4) and either ethylmorphine–HCl (10 μmoles), aminopyrine (18 μmoles) or aniline (5 μmoles) in a final volume of 3.0 ml. Semicarbazide–HCl (25 μmoles) served as a trapping agent for formaldehyde produced from ethylmorphine and aminopyrine. Samples were incubated for 15 min at 37° under air. Benzo(a)pyrene hydroxylase activity was assayed as described by Nebert and Gelboin [9], using authentic 3-hydroxybenzo(a)pyrene as standard. Samples were incubated at 37° under air for 8 min. Samples were read against appropriate tissue blanks and standards. All enzyme assays were optimized in each species with respect to protein concentration and time of incubation. Group means were compared statistically using Duncan's multiple range test.

Hepatic microsomal protein concentrations were similar in lesser bushbabies (male and female), greater bushbabies (male) and rats (male) but somewhat lower in tree shrews (male and female) (Table 1). Cytochrome P-450 levels in the greater bushbaby also approximated those in the rat and were significantly greater than concentrations in lesser bushbabies and tree shrews. No species differences in NADPH-cytochrome *c* reductase activity were demonstrable. Similarly, neither cytochrome P-450 content nor NADPH-cytochrome *c* reductase activity was sex-dependent in the lesser bushbaby or tree shrew.

The rates of metabolism of ethylmorphine and aminopyrine were far lower in lesser bushbabies and tree shrews than in rats and greater bushbabies, paralleling species differences in cytochrome P-450 concentrations. In contrast, aniline hydroxylase activity was similar in the lesser bushbaby, greater bushbaby and rat, but significantly lower in the tree shrew. Species differences in benzo(a)pyrene metabolism presented yet another pattern. Activity was lower in male and female lesser bushbabies than in the other species studied.

In the lesser bushbaby, no sex differences in drug-metabolizing activity were demonstrable for any of the substrates employed. The rates of ethylmorphine and aminopyrine demethylation were also similar in male and female tree shrews. However, both aniline and benzo(a)pyrene were metabolized more rapidly by livers obtained from female tree shrews than from males. Thus, sex differences in xenobiotic metabolism in the tree shrew differ considerably from those in the rat. In rats, ethylmorphine, aminopyrine and benzo(a)pyrene are metabolized far more rapidly by males than by females and aniline metabolism is not sex-dependent [10, 11]. Our observations are consistent with those of Litterst *et al.* [12] who also found that the rates of hepatic drug metabolism in tree shrews were greater in females than in males for some substrates, including benzo(a)pyrene.

These observations indicate that divergent patterns of hepatic drug metabolism exist among even closely related lower primate species. Both qualitative and quantitative differences have been demonstrated. At least some of the species differences in drug-metabolizing activity may be related to varying cytochrome P-450 concentrations. Hepatic microsomes from

the greater bushbaby, for example, have a far higher concentration of cytochrome P-450 than those from lesser bushbabies and tree shrews, which may account for the differences in aminopyrine and ethylmorphine demethylase activities. However, aniline hydroxylase activity in the lesser bushbaby and benzo(a)pyrene hydroxylase activity in the tree shrew are similar to activities in the greater bushbaby. Thus, species differences in the catalytic properties of some mixed function oxidases are also indicated. The high rates of hepatic benzo(a)pyrene metabolism (relative to other substrates) in the tree shrew have been noted previously by Freudenthal *et al.* [13] who suggested that tree shrews, therefore, might serve as good models for studies on chemical carcinogenesis. It is known that tree shrews are highly sensitive to the carcinogenic effects of polycyclic aromatic hydrocarbons [14].

The mechanism(s) responsible for the sex differences in drug metabolism in tree shrews is (are) unknown. Neither microsomal cytochrome P-450 levels nor NADPH-cytochrome *c* reductase activity was sex-dependent. Since both androgens and estrogens contribute to sex differences in xenobiotic metabolism in rats [15], the effects of gonadal hormones on hepatic mixed function oxidases in the tree shrew should be examined. Further studies are also needed to determine the molecular basis for the divergent patterns of drug-metabolizing activity among these closely related prosimian species. Such studies may also provide valuable information concerning the suitability of using prosimians as experimental models for evaluating drug metabolism and toxicity in man.

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Table 1. Hepatic drug metabolism in the greater bushbaby (*Galago crassicaudatus*), the lesser bushbaby (*Galago senegalensis*) and the tree shrew (*Tupaia glis*)*

	Microsomal protein (mg/g liver)	Cytochrome P-450 (nmoles/mg protein)	NADPH-cytochrome *c* reductase (nmoles/min × mg protein)	Drug metabolism (nmoles product/min × g liver)			
				Ethylmorphine	Aminopyrine	Aniline	Benzo(a)pyrene
Lesser bushbaby							
Male (12)	25.5 ± 1.0	0.47 ± 0.06†	108.9 ± 11.3	148.9 ± 12.3†	190.9 ± 23.7†	34.3 ± 4.3	6.2 ± 0.8†
Female (10)	25.0 ± 1.5	0.40 ± 0.02†	114.8 ± 12.3	111.0 ± 11.2†	222.0 ± 17.9†	31.0 ± 3.1	6.7 ± 1.0†
Greater bushbaby							
Male (6)	29.8 ± 2.2	1.02 ± 0.11	87.3 ± 9.2	308.6 ± 12.8	379.6 ± 32.7	35.4 ± 4.1	19.7 ± 1.6
Tree shrew							
Male (9)	21.7 ± 1.8†	0.31 ± 0.05†	84.9 ± 6.9	118.8 ± 11.8†	180.4 ± 22.2†	10.7 ± 1.9‡, §	14.8 ± 3.1 ‡
Female (8)	22.9 ± 2.3†	0.36 ± 0.03†	87.3 ± 9.1	136.1 ± 15.1†	176.7 ± 15.2†	19.5 ± 1.9§	24.3 ± 2.8
Rat							
Male (10)	28.3 ± 1.8	0.89 ± 0.07	104.2 ± 9.8	376.4 ± 29.8	423.7 ± 37.3	31.8 ± 2.7	20.8 ± 1.9

* Values are expressed as means ± S.E.; the number of animals per group is indicated in parentheses.
† $P < 0.05$ (vs greater bushbaby and rat). ‡ $P < 0.05$ (vs female of same species). § 0.05 (vs all other species).

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